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Coupling chromosomal replication to cell growth by the initiator protein DnaA in *Escherichia coli*

Qing Zhang, Hualin Shi*

State Key Laboratory of Theoretical Physics, Institute of Theoretical Physics, Chinese Academy of Sciences, Beijing 100190, China

HIGHLIGHTS

- Study how bacteria control replication initiation to match to cell growth.
- Get the replication initiation probability based on distribution of DnaA boxes.
- Propose a quantitative dynamic model to describe oscillation of DnaA.
- Regulating DnaA links up chromosomal replication with cell growth.
- RNA polymerase harmonizes chromosomal replication and cell growth.

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ABSTRACT

The initiation of chromosomal replication is strictly controlled during the cell cycle. Its frequency needs to be well-matched to the proliferation rate. In many bacteria, DnaA is the critical mediator in the regulation of replication initiation. In this work, the initiation probability is deduced based on the distribution of DnaA boxes at *oriC* in *Escherichia coli*. Taking into account more details, we develop a dynamic model to describe the oscillation of DnaA accompanied with the cell cycles. Our simulations show that the regulation of DnaA couples chromosomal replication to cell growth. We also discuss effects of other factors on DnaA oscillation. We propose that RNA polymerase is one of the candidates for harmonizing chromosomal replication and cell growth by adjusting *dnaA* transcriptional activity.

1. Introduction

Like most bacteria, *Escherichia coli* in proliferation should perform three essential tasks: cell growth, chromosomal replication and cell division. The bacterial growth depends on external conditions. In a more nutrient-rich environment, bacteria grow faster. In order to grow normally, the chromosomal replication should match the cell growth and proliferation no matter what the environment is. The replication proceeds from the origin (*oriC*) bidirectionally to the terminus (*ter*), and takes about 40 min for *E.coli* at 37 °C in a wide range of growth rates (Helmstetter and Cooper, 1968). The initiation of DNA replication occurs once and only once during one cell cycle, and many factors are responsible for its control. A question then arises: how does cell control DNA replication initiation to match the growth/proliferation rate as the environment changes?

During the past 30 years, many factors have been found to relate to the control of replication initiation. The most important factor is the initiator protein DnaA, which is highly conserved (reviewed in Skarstad and Boye, 1994; Messer, 2002; Kaguni, 2006; Mott and Berger, 2007). A number of other factors are involved in regulating the initiation of chromosomal replication in *E.coli* (reviewed in Kaguni, 2006; Kato, 2005; Katayama et al., 2010). Some essential information about the replication initiation are listed here:

1. DnaA can bind with ATP or ADP tightly, and both ATP-bound and ADP-bound forms of DnaA can bind to *oriC*, but only the ATP-bound form can initiate the DNA replication (Sekimizu et al., 1987). The transcription of DnaA can be repressed by its ATP-bound or ADP-bound form, but mainly by ATP-bound form (Speck et al., 1999; Atlung et al., 1985; Braun et al., 1985; Kücherer et al., 1986). A newly synthesized DnaA protein most likely binds ATP, since ATP is much more abundant in a cell (Speck et al., 1999) and also DnaA has a higher binding affinity with ATP (Sekimizu et al., 1987).

* Corresponding author. Tel.: +86 10 62582506.
E-mail address: shihl@itp.ac.cn (H. Shi).

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2. SeqA and Dam are cooperatively in charge of the eclipse period, during which re-initiation of replication is forbidden (Lu et al., 1994; Hiraga et al., 1998; Torheim and Skarstad, 1999; Freiesleben et al., 2000; Løbner-Olesen et al., 2005). After duplicated, *oriC* segments will remain in the hemimethylated state for a period. Then SeqA can directly bind to hemimethylated GATC sites at *oriC* to inhibit new initiation until *oriC* segments are fully methylated (Lu et al., 1994; Hiraga et al., 1998; Torheim and Skarstad, 1999; Freiesleben et al., 2000; Løbner-Olesen et al., 2005).
3. A lot of nonfunctional DnaA boxes, which are not directly involved in opening complex conformation at *oriC*, distribute on the chromosome (Roth and Messer, 1998; Nozaki et al., 2009), especially at the datA locus. This locus is a segment of 1 kb, which is located much closer to *oriC* than the replication terminus, and can bind hundreds of DnaA molecules (Kitagawa et al., 1996; Ogawa et al., 2002). These nonfunctional DnaA boxes play an important role in titrating DnaA (Hansen et al., 1991; Browning et al., 2004).
4. The Hda-clamp complex at least consists of Hda protein and β-subunit sliding clamp of DNA polymerase(pol) III holoenzyme. It hydrolyzes DnaA-ATP into DnaA-ADP along with chromosomal replication. This is usually called "regulatory inactivation of DnaA (RIDA)" (Katayama et al., 1998; Kurokawa et al., 1999; Kato and Katayama, 2001).
5. DnaA-ADP can be rejuvenated into DnaA-ATP spontaneously, but the process is extremely slow. The membrane acidic phospholipids and specific genomic sequences DARS can accelerate this process (Boeneman and Crooke, 2005; Fujimitsu et al., 2009).

Based partly on the above information, some theoretical models have been proposed (e.g., Hansen et al., 1991; Browning et al., 2004; Sompayrac and Maaløe, 1973; Mahaffy and Zyskind, 1989). Most of them succeeded in simulating the replication initiation coupled with the cell cycle. In this study, we develop a new dynamic model containing more details, which is illustrated in Fig. 1. RNA polymerase (RNAP) is a growth-rate-dependent global regulator (Klumpp and Hwa, 2008). We clarify how the initiation of DNA replication is coupled with the cell cycle at different growth rates by adjusting the free RNAP concentration. We simulate the dynamic model of chromosomal replication initiation at different growth rates. We discuss the effects of parameters involved in this model, and investigate how DnaA and some other factors cooperatively adjust the replication initiation to match the cell growth. We suggest that RNAP is the factor that makes the chromosomal replication coupling with the cell growth by tuning the DnaA oscillation.

2. Model

Our model is constructed based on previous models (reviewed in Mahaffy and Zyskind, 1989; Browning et al., 2004). In analogy with references (Mahaffy and Zyskind, 1989; Browning et al., 2004), we take into account processes of DnaA synthesis, DnaA autorepression in transcription, DnaA titration by DnaA boxes, DnaA inactivation, DnaA reactivation and replication initiation controlled by DnaA. Both DnaA-ATP and DnaA-ADP can bind to general DnaA boxes, the competition between DnaA-ATP and DnaA-ADP exists not only in the titration process at nonfunctional DnaA boxes, but also in binding process at *oriC*. Some previous models considered the former competition (e.g., Browning et al., 2004). With more up-to-date experimental data of Speck et al. (1999), we take into account the both competitive processes and also the negative role of DnaA-ADP in unwinding *oriC*. On the transcription of DnaA, we consider not only the effect of the concentration of free RNAP, but also the self-cooperative repression of DnaA-ATP. The nonfunctional DnaA boxes are divided into two classes, those at *datA* locus and those scattered in the chromosome, to distinguish their roles in controlling replication initiation.

2.1. Replication initiation probability

DNA replication initiates at the genetically unique origin (*oriC*) in *E.coli*. The *oriC* has total length of 245 bp, including five 9-bp DnaA recognition sites termed as R-boxes (R1–R4 and R5M) (Messer, 2002; Fuller et al., 1984; Matsui et al., 1985), and some other DnaA binding sites termed as I-sites (McGarry et al., 2004). R-boxes can bind both DnaA-ADP and DnaA-ATP with a similar affinity (Sekimizu et al., 1987), whereas I-sites can bind DnaA-ATP much more tightly than DnaA-ADP (McGarry et al., 2004; Speck and Messer, 2001). DnaA boxes of *oriC* can be grouped into two classes according to their binding affinities: (i) the strong ones, which include R1, R2 and R4; (ii) the weak ones, which include R5M, R3, I1, I2 and I3 (Margulies and Kaguni, 1996; Leonard and

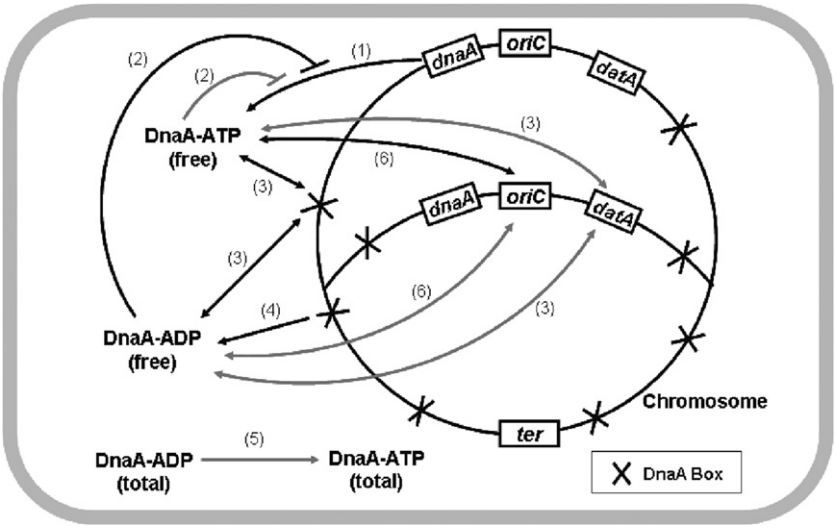

Fig. 1. Schematic illustration for our model. It is adapted from Browning et al. (2004), Mahaffy and Zyskind (1989). (1) Newly synthesized DnaA is bound with ATP. (2) Free DnaA-ATP and free DnaA-ADP repress the transcription of DnaA. (3) DnaA boxes titrate DnaA-ATP and DnaA-ADP. (4) Bound DnaA-ATP is hydrolyzed into free DnaA-ADP. (5) DnaA-ATP regenerates from DnaA-ADP. (6) Free DnaA-ATP and free DnaA-ADP compete in binding to *oriC*. The gray lines indicate the processes which we address differently from the earlier models.

Grimwade, 2005; Miller et al., 2009). Before the replication is initiated, DnaA-ATPs assemble into an ordered multimeric pre-replication complex (pre-RC). This complex will unwind *oriC* (Leonard and Grimwade, 2005; Miller et al., 2009) and help replicative DNA helicase (DnaB) load on the newly exposed single strands (Fang et al., 1999).

In order to derive the condition for the replication initiation, we introduce the "replication initiation probability" as the chance for most of DnaA boxes at *oriC* to be bound by DnaA-ATPs at certain concentrations of DnaA-ATP and DnaA-ADP. In the following, we will deduce the replication initiation probability used in our model.

We denote the concentration of free DnaA-ATP(DnaA-ADP) by c_1 (c_2), the probability of one DnaA box binding with one DnaA-ATP(DnaA-ADP) by p_1 (p_2). We then have

$$k^*_{on1}(1-p_1-p_2)c_1 = k^*_{off1}p_1, \tag{1}$$

$$k^*_{on2}(1-p_1-p_2)c_2 = k^*_{off2}p_2, \tag{2}$$

where k^*_{on1}, k^*_{on2}, k^*_{off1} and k^*_{off2} are the corresponding binding and unbinding rates, respectively. It is straightforward to get

$$p_1 = \left(1+\frac{K_1}{c_1}+\frac{K_1/K_2}{c_1/c_2}\right)^{-1}, \tag{3}$$

where $K_1 = k^*_{off1}/k^*_{on1}$ and $K_2 = k^*_{off2}/k^*_{on2}$ are the corresponding dissociation constants.

We simplify *oriC* (Miller et al., 2009) as shown in Fig. 2. The simplified *oriC* has three identical strong DnaA boxes and four identical weak DnaA boxes. The strong DnaA box can bind both DnaA-ATP and DnaA-ADP (with dissociation constants K_1 and K_2, respectively). The weak DnaA box can bind DnaA-ATP (with dissociation constant K_1) only if its nearest strong box has been occupied by DnaA-ATP. All these strong DnaA boxes will be occupied by DnaA-ATP with probability $(1+K_1/c_1+(K_1/K_2)/(c_1/c_2))^{-3}$. The probability for the weak DnaA boxes all to be occupied is $(1+K_1/c_1)^{-4}$. We suppose that occupancy of all the seven DnaA boxes by DnaA-ATP will lead more molecules of DnaA-ATP to form the ordered multimeric pre-RC, and then the duplex unwinding region (DUE) will be opened. So the condition for replication initiation can be defined simply as "all the seven DnaA boxes are occupied by DnaA-ATP", and the probability for replication initiation is then written as

$$P_{ini} = \left(1+\frac{K_1}{c_1}+\frac{K_1/K_2}{c_1/c_2}\right)^{-3}\left(1+\frac{K_1}{c_1}\right)^{-4}. \tag{4}$$

If $K_1/K_2 \ll 1$, then $P_{ini} \simeq (1+K_1/c_1)^{-7}$. In this case, only the concentration of DnaA-ATP determine the initiation, it is called noncompetition initiation. If $c_1 \gg K_1$ and $c_2 \gg K_2$, we have $P_{ini} \simeq (1+(K_1/K_2)/(c_1/c_2))^{-3}$. This is the case of the full competition initiation, where the ratio between the concentrations of DnaA-ATP and DnaA-ADP determine the initiation. In *E.coli*, both K_1 and K_2 are about 1 μm^{-3} (Schaper and Messer, 1995), c_1 and c_2 are much larger than 1 μm^{-3} (see the Results). In general, the chromosomal replication of *E.coli* is in the full competition initiation regime.

2.2. Dynamic model for DnaA-mediated replication initiation

In this study, we propose a dynamic model to simulate how a cell couples the chromosomal replication to the cell cycle by regulating DnaA. We denote the total numbers of DnaA-ATP and DnaA-ADP by x and y, those of the free DnaA-ATP and DnaA-ADP by x_f and y_f, and those of the bound DnaA-ATP and DnaA-ADP by x_b and y_b, respectively. The interval between two successive cell divisions gives a cell cycle. Doubling time is denoted by T, and growth rate (i.e. doubling rate) is defined as $\mu = 1/T$. Replication time C and division time D are set to be 40 min and 20 min, respectively (Helmstetter and Cooper, 1968). The growth manner of the cell size during one cell cycle is still unknown (Neidhardt et al., 1990). Both linear mode $V(t) = V_0(1+\mu t)$ and exponential mode $V(t) = V_0 2^{\mu t}$ are used in our simulations, with $V_0 = V_{00}2^{\mu(C+D)}$ (V_{00} is the minimum newborn volume; we assume that the mass per volume is independent of the growth rate) and $t \in [0,T)$ (Donachie, 1968). Since the two modes for size increment do not show any significant difference, we just present the results obtained with the linear mode.

Based on the work of Speck et al. (1999), we assume that the repression of DnaA-ATP and DnaA-ADP on DnaA transcription takes the form of $1/(1+(k_{f1}x_f/V(t))^2+k_{f2}y_f/V(t))$, where k_{f1} and k_{f2} are the binding constants of DnaA-ATP and DnaA-ADP, respectively, and the factor 2 comes from self-cooperation of DnaA-ATP. Fitting the experimental data (Speck et al., 1999), we obtain $k_{f1} = 0.024\ \mu m^3$ and $k_{f2} = 0.0135\ \mu m^3$ (shown in Fig. 3). Since *dnaA* promoter is weak, we propose that the transcriptional activity of DnaA is proportional to the concentration of free RNAP (Hansen et al., 1982). The rate of DnaA synthesis is then expressed as

$$\rho(t) = \frac{\alpha n_{ori}(t)[RNAP]_f}{1+(k_{f1}x_f(t)/V(t))^2+k_{f2}y_f(t)/V(t)}, \tag{5}$$

where α is the synthesis constant, n_{ori} is the number of replication origin and $[RNAP]_f$ is the concentration of free RNAP.

It has been shown that DnaA-ADP can be rejuvenated into DnaA-ATP either in membrane-dependent (Boeneman and Crooke, 2005) or DARS-dependent ways (Fujimitsu et al., 2009), but the details are not clear. We assume DnaA reactivation rate $\sigma(t) = \beta y(t)$, where β is the reactivation rate constant.

There are a large number of nonfunctional DnaA boxes, some locate in *datA* and the others distribute elsewhere on chromosome. We denote the total number of nonfunctional DnaA boxes by n_{box}, those in *datA* by n_{box1} and the others by n_{box2}. For convenience, we assume that DnaA boxes distribute homogeneously on the chromosome except *datA*, and *datA* is duplicated at the time of initiation. We use n_{fork} and n_{ori} to indicate the numbers of replication fork and

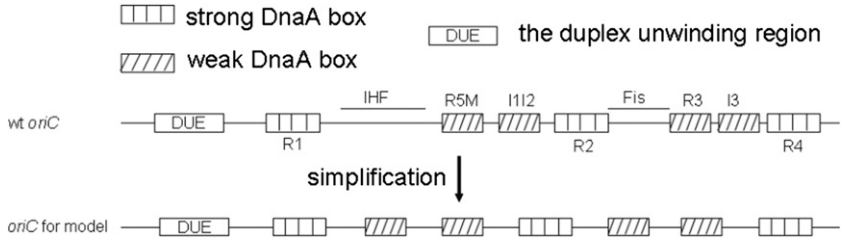

Fig. 2. Structure characteristics of *E.coli oriC*. In WT *oriC* (adapted from Miller et al., 2009), positions of DnaA boxes, binding sites of Fis and IHF, and the duplex unwinding region (DUE) are indicated. Fis and IHF can affect the binding of DnaA with DnaA boxes (Margulies and Kaguni, 1996; Leonard and Grimwade, 2005; Miller et al., 2009). We regard their roles as part of background of DnaA-ATP oligomer formation at *oriC*, and then a simplified oriC is obtained. The simplified oriC has three identical strong DnaA boxes (originated from R1, R2 and R4, respectively) and four identical weak boxes (originated from R5M, I1I2, R3 and I3, respectively). DnaA-ATP and DnaA-ADP compete in binding with the strong boxes. DnaA-ATPs bound with strong boxes direct binding of DnaA-ATP with adjacent weak boxes.

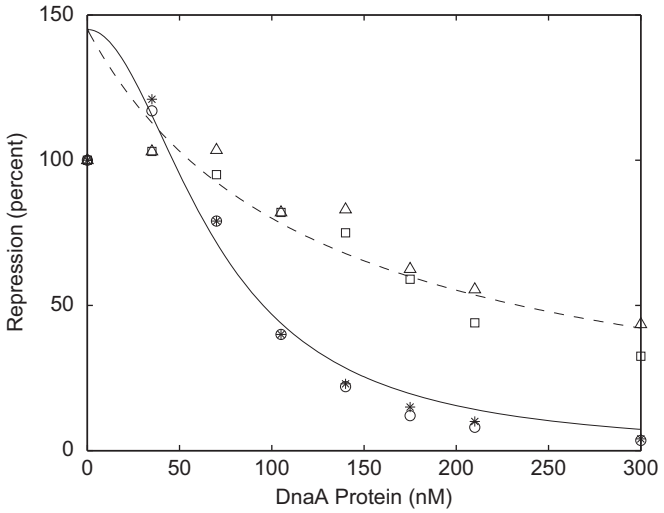

Fig. 3. Fitting the experimental data of Speck et al. (1999) about the repression of DnaA-ATP and DnaA-ADP on DnaA transcription. *E.coli dnaA* transcription unit contains two promoters: *dnaAp1* and *dnaAp2*, both of which can be repressed by DnaA-ATP and DnaA-ADP (reviewed in Speck et al., 1999). '*': data for DnaA-ATP repressing *dnaAp1*, '○': data for DnaA-ATP repressing *dnaAp2*, '△': data for DnaA-ADP repressing *dnaAp1*, '□': data for DnaA-ADP repressing *dnaAp2*. The fitting function is $a/(1+(k_{f1}[DnaA{-}ATP])^2+k_{f2}[DnaA{-}ADP])$ and the parameters fitted to the data are: $a=145$, $k_{f1}=0.024\ \mu m^3$ and $k_{f2}=0.0135\ \mu m^3$. Solid line denotes the fitting result to average repression of DnaA-ATP on *dnaAp1* and *dnaAp2*; dashed line denotes the fitting result to average repression of DnaA-ADP on *dnaAp1* and *dnaAp2*.

origin, n^*_{box1} and n^*_{box2} to indicate the number of nonfunctional DnaA boxes in one copy of *datA* and elsewhere in one chromosome. All n_{box}, n_{box1}, n_{box2}, n_{fork} and n_{ori} will change with DNA replication. The dynamic process is represented by

$$dn_{ori}/dt = n_{ori}(t_{ini}^-)\delta(t-t_{ini}), \tag{6}$$

$$dn_{fork}/dt = 2n_{ori}(t_{ini}^-)(\delta(t-t_{ini})-\delta(t-t_{ini}-C)), \tag{7}$$

$$n_{box1}(t) = n^*_{box1}\cdot n_{ori}(t), \tag{8}$$

$$dn_{box2}(t)/dt = \frac{1}{2C}\cdot n^*_{box2}\cdot n_{fork}(t), \tag{9}$$

$$n_{box}(t) = n_{box1}(t)+n_{box2}(t), \tag{10}$$

where t_{ini} indicates the time of replication initiation, t_{ini}^- indicates that t goes to t_{ini} from left, and $\delta(x)$ is the Dirac delta function. As DnaA-ATP is hydrolyzed along with DNA replication (Kurokawa et al., 1999; Kato and Katayama, 2001), we write DnaA inactivation rate as

$$\epsilon(t) = \frac{x_b}{n_{box}}\frac{dn_{box}}{dt} = \frac{x_b}{n_{box}}\left(n_{box1}(t_{ini}^-)\delta(t-t_{ini})+\frac{1}{2C}\cdot n^*_{box2}\cdot n_{fork}(t)\right). \tag{11}$$

The variation of total numbers of DnaA-ATP and DnaA-ADP are determined by DnaA synthesis, inactivation and reactivation:

$$\frac{dx(t)}{dt} = \rho(t)+\sigma(t)-\epsilon(t), \tag{12}$$

$$\frac{dy(t)}{dt} = -\sigma(t)+\epsilon(t). \tag{13}$$

Since DnaA molecule transforms between the bound and unbound state very rapidly (Schaper and Messer, 1995), we suppose that the process of DnaA titration is much faster than DNA replication, cell size increasing and DnaA activity transformation. So the titration process can be described with a much smaller time scale by

$$\frac{dx_f(t')}{dt'} = -k_{on1}x_f(t')(n_{box}(t)-x_b(t')-y_b(t'))/V(t)+k_{off1}x_b(t'), \tag{14}$$

$$\frac{dy_f(t')}{dt'} = -k_{on2}y_f(t')(n_{box}(t)-x_b(t')-y_b(t'))/V(t)+k_{off2}y_b(t'), \tag{15}$$

$$x_b(t') = x(t)-x_f(t'), \tag{16}$$

$$y_b(t') = y(t)-y_f(t'), \tag{17}$$

where k_{on1} (k_{off1}) is the binding (unbinding) rate of DnaA-ATP to (from) the nonfunctional DnaA box, and k_{on2} (k_{off2}) is that for DnaA-ADP. Notice that we have neglected the repression of SeqA on DnaA transcription and titration by nonfunctional DnaA boxes.

2.3. Simulations

With the above model, we simulate the time evolution of DnaA coupled with cell growth. Some details in the simulation are:

1. The simulation is performed using Euler forward algorithm with time steps $\Delta t = 10$ s for Eqs. (10)–(11) and $\Delta t' = 0.1$ s for Eqs. (12)–(15). Even if we use much smaller time steps or fourth-order Runge–Kutta algorithm, no obvious difference is observed. When the initiation probability exceeds the threshold (0.3) and also it is not in the eclipse period, replication is initiated. The eclipse period is supposed to be 10 min (Hansen et al., 1991; Campbell and Kleckner, 1990).
2. We assign arbitrary initial values to x, y, x_f, y_f and T, and suppose that the initial DNA content Gc is one genome equivalent, $n_{ori}(0)=1$, $n_{fork}(0)=0$, $n_{box1}(0)=n^*_{box1}$, $n_{box2}(0)=n^*_{box2}$ and $n_{box}(0)=n^*_{box1}+n^*_{box2}$.
3. When cell division occurs, x, y, x_f, y_f, n_{ori}, n_{fork}, n_{box1}, n_{box2}, n_{box} and Gc are halved, T is reset based on several (e.g., five) former cycles, μ and V_0 are updated according to $\mu=1/T$ and $V_0=V_{00}2^{\mu(C+D)}$, and then a new cell cycle begins.
4. The simulation will continue until a regular oscillation state arrives or the running time goes beyond the limit.
5. Some parameters in the model are selected properly based on literatures: $V_{00}=0.5\ \mu m^3$ (Churchward et al., 1981); $k_{on1}=3\times10^{-5}\ \mu m^3/s$, $k_{on2}=2\times10^{-5}\ \mu m^3/s$, $k_{off1}=0.003\ s^{-1}$, $k_{off2}=0.003\ s^{-1}$ (Schaper and Messer, 1995); $n^*_{box1}=350$ (Kitagawa et al., 1996), $n^*_{box2}=300$ (Roth and Messer, 1998; Ogawa et al., 2002); $K_1=K_2=1\ \mu m^{-3}$ (Schaper and Messer, 1995). Others are chosen by fitting: $\alpha=0.0005\ \mu m^3/s$, $\beta=0.00015\ s^{-1}$.

In simulation, we take free RNAP concentration as an adjustable parameter. Through adjusting it, we can obtain different initiation frequencies or growth rates.

3. Results

3.1. The oscillations coordinated with cell cycle

If a proper value is assigned to $[RNAP]_f$, we obtain periodic oscillation of DnaA, which are well-matched to the normal cell cycle with a certain growth rate (see Figs. 4 and 5). Start from arbitrary initiation condition, levels of all forms of DnaA finally oscillate regularly in our simulations (see Fig. 4). The regular DnaA oscillations result in normal initiation of chromosomal replication, which is coupled well to cell growth and cell division (see Fig. 5). Shortly after the initiation of DNA replication, the replication fork passes through *datA* locus and then the DnaA-ATPs bound there are hydrolyzed into DnaA-ADPs. So the number

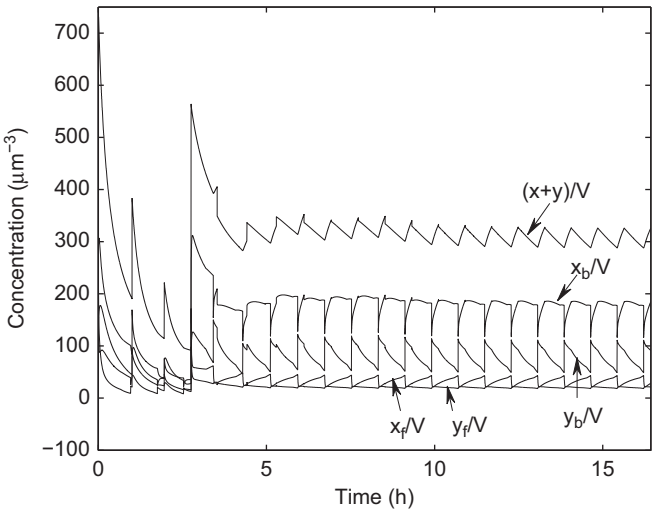

Fig. 4. Change of DnaA concentration (x_f/V, y_f/V, x_b/V, y_b/V, or $(x+y)/V$) with time turn into regular and periodic oscillation from the irregular form of a beginning period. $[RNAP]_f$ is chosen as 200 μm^{-3}.

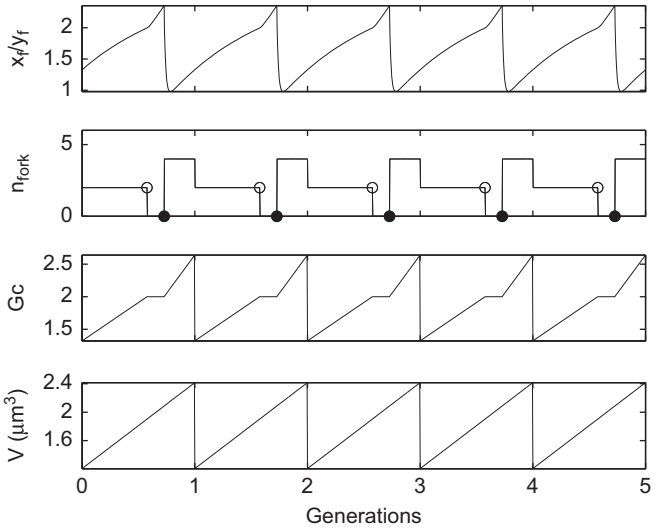

Fig. 5. When DnaA oscillation in Fig. 4 becomes regular and periodic, DNA replication is well-coupled with the cell cycle. From up to down, it shows changes of free DnaA-ATP/free DnaA-ADP (x_f/y_f), number of replication forks (n_{fork}), genome content (Gc) and cell volume (V) during cell cycles. Filled circles indicate replication initiations, open circles indicate replication terminations. Unit for Gc is genome equivalent per cell. The abscissa is represented by generations instead of time and the doubling time is about 0.79 h.

of DnaA-ATP decreases, and the number of DnaA-ADP increases. At the same time, the copy number of *datA* is doubled and the number of DnaA boxes increases. This will reduce the number of free DnaA-ATP by the fast titration process. Thus, the ratio of free DnaA-ATP and DnaA-ADP is reduced further. As the synthesis and the reactivation of DnaA overcome the inactivation of DnaA, the concentration of free DnaA-ATP becomes rising gradually in contrast to free DnaA-ADP. When the eclipse period is passed and the initiation condition is satisfied, one new round of DNA replication begins.

It is also indicated that free DnaA-ATP concentration, total free DnaA concentration, and (total DnaA-ATP)/(total DnaA-ADP) all change with time in a similar way to (free DnaA-ATP)/(free DnaA-ADP). When DNA replication initiates, all of them reach their maximum. It seems that we can even set the replication initiation condition as "one of them exceeds a threshold". This means that different initiation conditions used in the previous models are equivalent in some sense.

In conclusion, the initiation of chromosomal replication is well coordinated with the cell cycle through the regulation on the relative level of DnaA-ATP and DnaA-ADP in *E.coli*.

3.2. Growth rate dependences of biological quantities

Selecting different free RNAP concentrations in our simulation, we obtain replication initiation frequencies which are corresponding to different growth rates (see Fig. 6). In normal environmental conditions, faster bacterial growth requires a higher concentration of free RNAP (Klumpp and Hwa, 2008). Our results are agreement with this point. We also derive the dependences of other biological quantities on the growth rate by calculating their averages in one cell cycle at different growth rates.

The numbers of total DnaA per cell at different growth rates obtained from our simulation are close to those from experiments (Chiaramello and Zyskind, 1989; see Fig. 7a). The average concentrations of DnaAs in different forms increase several folds as the growth rate increase in a slow-growth region (0.5 to $\sim$1.5 doublings/h), whereas increase very little in a fast-growth region ($\sim$1.5−3 doublings/h) (see Fig. 7b). This is similar to that relationship between free RNAP concentration and growth rate shown in Fig. 6.

In a fast-growing cell (doubling time < 40 min), the initiation of chromosomal replication is so frequent that one new round of replication must be initiated before the previous round has finished. It is inevitable that "multifork replication" pattern arises (Cooper and Helmstetter, 1968; see Fig. 8). Helmstetter and Cooper (1968) provided the rates of DNA synthesis during the cell cycle of *E.coli* based on their own experiments. By simulation, we get the numbers of replication forks during the cell cycle at different growth rates (see Fig. 8). The rate of DNA synthesis is proportional to the number of replication forks. Our results are consistent with those of Helmstetter and Cooper (1968). As shown in Fig. 8, if doubling time $T > C+D$ (i.e., $T > 1$ h), the initiation and the termination in a round of replication occur in the same cell cycle and the fork number takes the pattern $0 \to 2 \to 0$. If $(C+D)/2 < T < C+D$ (i.e., 0.5 h < T < 1 h), the initiation occurs in one cell cycle, whereas the termination occurs in

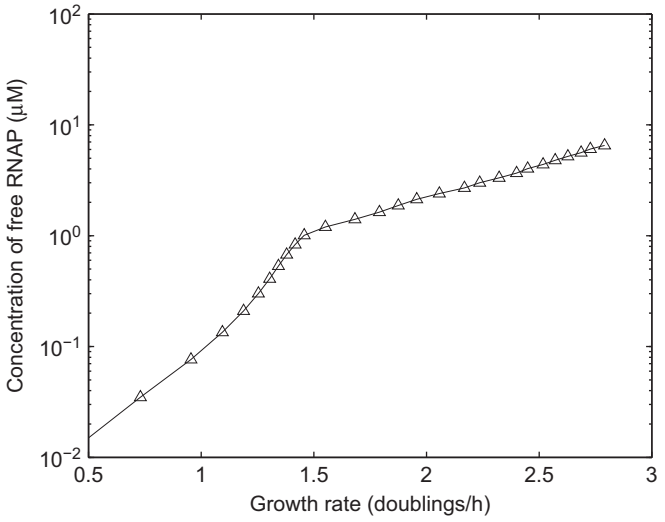

Fig. 6. Free RNAP concentration increases with growth rate (replication initiation frequency). The growth rate is in unit of doublings/h and the free RNAP concentration is in unit of μM.

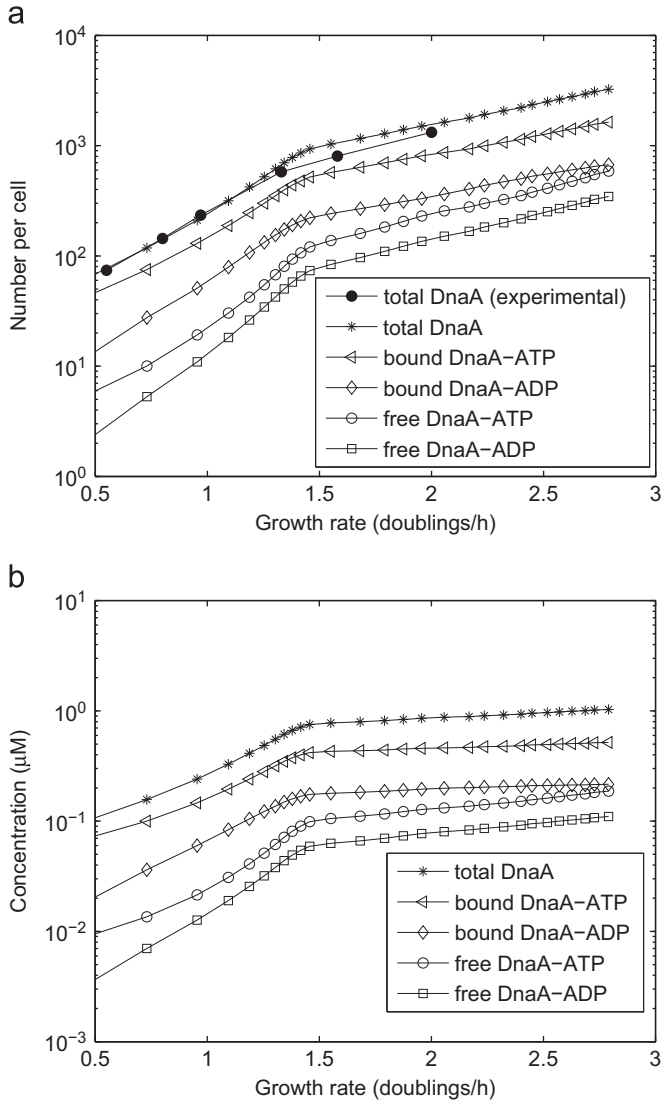

Fig. 7. Dependence of numbers (a) and concentrations (b) of all the forms of DnaA on growth rate. The experimental data are from Chiaramello and Zyskind (1989).

the next one, and the fork number takes the pattern $2\rightarrow0\rightarrow4$ or $2\rightarrow6\rightarrow4$. If $(C+D)/3<T<(C+D)/2$ (i.e., 0.33 h $<T<$ 0.5 h), the initiation occurs in the cell cycle before the last one which the termination occurs in, and the fork number takes the pattern $6\rightarrow4\rightarrow12$.

The simulations show that cell size, replication origin number, replication terminus number and DNA content increase with growth rate approximately in an exponential manner. This is in agreement with experimental result (Bremer and Dennis, 1996). Donachie (1968) defined "initiation mass" as the critical mass per origin when initiation occurs and concluded that initiation mass is invariant with growth rate. Under the assumption that cell density is independent on growth rate, initiation mass is reflected by initiation volume. Our results show that initiation volume is invariant (for exponential growth mode) or vary very little (for linear growth mode) with growth rate. It must be noted that this conclusion also depends on the assumption that the relation between newborn volume and growth rate takes the form of $V_0=V_{00}2^{\mu(C+D)}$.

It is clearly shown in Fig. 9 that the number of replication origin per unit volume nearly does not change, DNA content decreases, whereas the number of replication fork increases as growth rate increases. The titration effect on DnaA-ATP will be weakened due to the declining of DnaA box number and the increasing of DnaA-ATP hydrolysis rate per unit volume when growth rate increases. Then (free DnaA-ATP)/(free DnaA-ADP) and initiation probability will recover faster to their thresholds. The titration effect is much weakened over the range 0.5 to $\sim$1.5 doublings/h, but not so much over the range $\sim$1.5−3 doublings/h. These results are similar to the relationship between free RNAP concentration (or DnaA) and growth rate.

Based on our assumptions, the dependence of $n_{ori}(t)$, $n_{ter}(t)$, $n_{fork}(t)$ and $V(t)$ on μ can be obtained easily. We analytically derive the growth rate dependence of average numbers of the origin, the terminus, replication fork and cell volume, respectively (Appendix A). These results obtained by our numerical simulation are consistent perfectly with those analytic formulae.

3.3. Factors affecting the frequency of replication initiation

We get a different initiation frequency (growth rate) if we assign a different free RNAP concentration in our simulation. This relationship has been shown in Fig. 6. It is consistent with the model based on the partition of RNAP (Klumpp and Hwa, 2008). As a growth-rate-dependent global regulator, RNAP is the most possible player that coordinate initiation frequency of chromosomal replication and growth rate by adjusting *dnaA* transcriptional activity.

By simulation, we also investigate the effects of the other parameters in our model. The results are listed below:

1. The frequency of replication initiation increases as α, β and k_{off1}/k_{on1} increase, whereas it decreases as k_{f1}, k_{f2}, n^*_{box2} and k_{off2}/k_{on2} increase. This is in agreement with previous experiments. DnaA autorepression in transcription, the titration by DnaA boxes and the competition between DnaA-ADP and DnaA-ATP in titration process negatively regulate the replication initiation. However, DnaA synthesis and DnaA reactivation positively regulate the replication initiation (see the literatures in the Introduction). It is also shown that the effect of n^*_{box1} is small, which suggests that *datA* may take the role of guaranteeing the high robustness of replication initiation.
2. When V_{00} is chosen smaller, all the growth rate, the initiation volume and the average volume become smaller. To clarify whether it is the cell mass that propels the initiation, we use growth-rate-independent newborn cell size in our simulation. The initiation frequency (i.e. growth rate) always increases with the free RNAP concentration. It seems that the replication initiation is affected but not determined by cell mass. Actually, some experiments have shown that initiation mass varies with the growth rate in *E.coli* (Churchward et al., 1981; Wold et al., 1994).
3. To explore the effects of C or D, we assume $V_0=4\,\mu m^3$ which is independent on C and D. The results show that the initiation frequency decreases as C or D increases.
4. The eclipse period does not affect initiation frequency. It means that the eclipse period is mainly play role in controlling the synchronization and the robustness of replication initiation.
5. As expected, when K_1, K_1/K_2 or the threshold for initiation probability is chosen larger, the initiation frequency become smaller.

In conclusion, chromosomal replication initiation is well-matched to bacterial growth by regulating the availability of free activated DnaA. As the environment is changed, initiation frequency may vary together with growth rate by adjusting free RNAP concentration.

4. Discussion

In our model, we consider the competition between DnaA-ATP and DnaA-ADP. We show that the competition and noncompetition

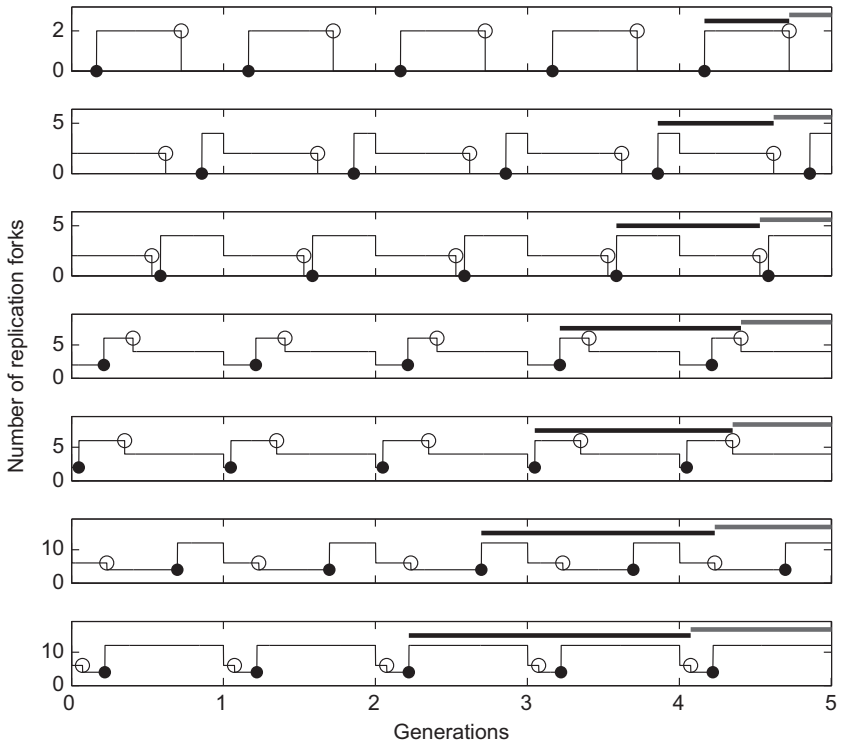

Fig. 8. The number of replication forks changes periodically with the cell generation at different growth rates. In each subfigure, filled circle, open circle, wider black line and wider gray line indicate replication initiation, replication termination, replication period C and division period D, respectively. From up to down, $[RNAP]_f$ is chosen as 30 μm^{-3}, 100 μm^{-3}, 500 μm^{-3}, 1000 μm^{-3}, 1300 μm^{-3}, 2000 μm^{-3} and 4000 μm^{-3}, respectively, and the doubling time is about 1.20 h, 0.88 h, 0.71 h,0.56 h, 0.51 h, 0.43 h and 0.36 h, respectively.

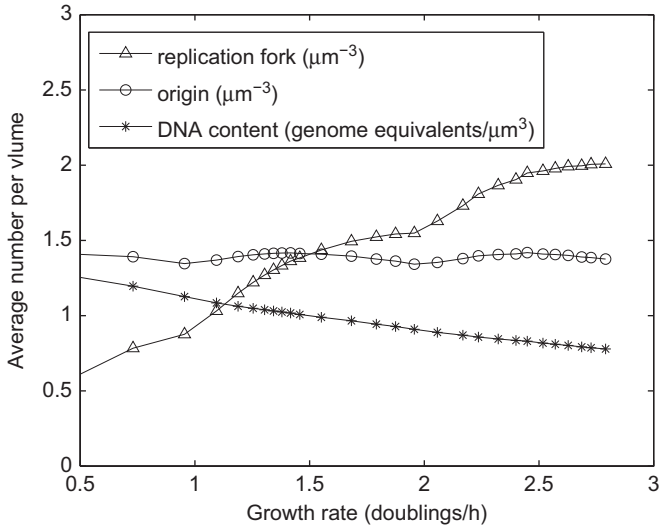

Fig. 9. Dependence of average numbers of replication fork, replication origin and DNA content per unit volume on growth rate.

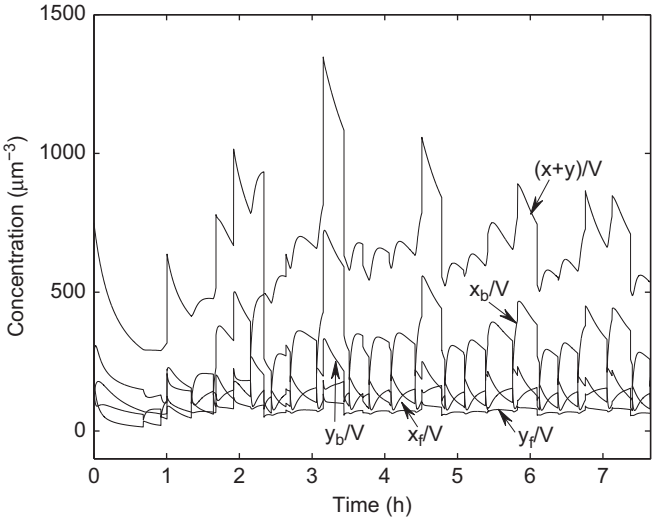

Fig. 10. The level of each form of DnaA does not arrive at a regular oscillation which is coupled to the cell cycle, where 5000 μm^{-3} is assigned to $[RNAP]_f$.

initiation modes are very different. Although the initiation probability increases with the DnaA-ATP concentration, it mainly depends on the ratio between the concentrations of DnaA-ATP and DnaA-ADP in competition mode. In order to get the same initiation probability, higher DnaA-ATP concentration is required in competition mode. That means the effects coming from DnaA-ATP fluctuation will be smaller. This may be the reason that *E.coli* has selected the competition mode in evolution.

The parameters in our model must be chosen in a proper way. Otherwise, we cannot get regularly periodic oscillations. If $[RNAP]_f$ exceeds the critical value, DnaA oscillations could not become regular and the cell cycle will be abnormal (e.g., see Fig. 10). We have tested our numerical algorithm and rule out the possibility of the irregular oscillation coming from computation error. We confirm that the reasonable oscillation can be obtained only when each biological quantity acquired by simulation is consistent with previous experimental data.

During the eclipse period, SeqA, Dam and some other factors take important roles in repressing re-initiation of the newly duplicated *oriC* (Hiraga et al., 1998; Torheim and Skarstad, 1999;

Freiesleben et al., 2000). They help chromosomal replication take place synchronously and robustly, and avoid the abnormal initiation (Hansen et al., 1991). Thus, the eclipse period is essential for normal DNA replication.

The parameters k_{f1}, k_{f2}, n^*_{box1}, n^*_{box2}, k_{off1}/k_{on1}, k_{off2}/k_{on2}, K_1, K_1/K_2 and V_{00} should have been fixed through billions years of evolution and they may have different values in different strains. In addition to the membrane and DARS, DnaA reactivation probably also need the help from some other growth-rate-dependent factors, such as cAMP (Hughes et al., 1988). So β may be growth-rate-dependent. The experiments also indicate that both C and D decrease with growth rate (Bremer and Dennis, 1996). If all these growth-rate dependences are taken into account, some corrections to the relationship between free RNAP and growth rate should be done.

Bacterial growth rate is determined by the environmental condition. Under stress conditions (e.g., amino acid starvation), guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp) accumulate rapidly and cause a stringent response (reviewed in Potrykus and Cashel, 2008). Some researchers have shown that (p)ppGpp inhibit replication initiation in *E.coli* (e.g., Chiaramello and Zyskind, 1990; Levine et al., 1991; Schreiber et al., 1995). Chiaramello and Zyskind (1990) showed that the concentration of (p)ppGpp is negatively correlated with both the DnaA transcriptional activity and the growth rate. They proposed a mechanism of coupling DNA replication to the bacterial growth rate. (p)ppGpp can inhibit the transcription of DnaA directly through destabilizing the promoter-RNAP open complexes during transcription initiation with the help of DksA or indirectly through altering the relative levels of σ factors (Srivatsan and Wang, 2008). So, it is possible that (p)ppGpp provides a coupling between replication initiation and growth rate in stringent response by regulating DnaA expression level.

Our model demonstrates that *E.coli* can coordinate chromosomal replication initiation with cell growth perfectly by regulating DnaA. Based on our simulations, we propose that it is mainly by adjusting the concentration of free effective RNAP. *E.coli* and other bacterial organisms, such as *Bacillus subtilis* and *Caulobacter crescentus*, have conserved DnaA and diverse *oriC* regulatory systems (Mott and Berger, 2007; Katayama et al., 2010) It is worth studying the similarity and the difference between the dynamic behaviors of these systems. DnaA reactivation is important in the regulation of replication initiation, but the detail is still not clear and worth exploring further. To some extent, C period and D period are also growth-rate-dependent (Bremer and Dennis, 1996). A question arises: how are they regulated and coupled to growth rate? Answering this question may help to further understand the cell cycle control in bacteria.

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Appendix A. The theoretical formulae for the growth-rate-dependence of several biological quantities

Based on the assumptions in our model, we can theoretically derive the dependences of the average numbers of chromosomal origins, terminuses and replication forks, the average volume and the initiation volume of one normal cell of *E.coli* on the growth rate.

1. The average numbers of chromosomal origins and terminuses can be derived by

$$\langle n_{ori}\rangle = \frac{1}{T}\int_0^T n_{ori}(t)\,dt = \begin{cases} (C+D)\mu+1, & T \ge C+D, \\ 2(C+D)\mu, & \frac{C+D}{2} \le T < C+D, \\ 4(C+D)\mu-4, & \frac{C+D}{3} \le T < \frac{C+D}{2}, \\ \cdots & \cdots \end{cases} \tag{A.1}$$

$$\langle n_{ter}\rangle = \frac{1}{T}\int_0^T n_{ter}(t)\,dt = D\mu+1, \tag{A.2}$$

respectively, and then the average number of replication forks can be obtained by

$$\langle n_{fork}\rangle = 2(\langle n_{ori}\rangle - \langle n_{ter}\rangle) = \begin{cases} 2C\mu, & T \ge C+D, \\ (4C+2D)\mu-2, & \frac{C+D}{2} \le T < C+D, \\ (8C+6D)\mu-10, & \frac{C+D}{3} \le T < \frac{C+D}{2}, \\ \cdots & \cdots \end{cases} \tag{A.3}$$

2. As the genome content (Gc) in one cell cycle is

$$Gc(t) = Gc(0) + \frac{1}{2C}\int_0^t n_{fork}(t')\,dt', \quad t \in [0,T] \tag{A.4}$$

we can obtain the average Gc through

$$\begin{aligned} \langle Gc(t)\rangle &= \frac{1}{T}\int_0^T Gc(t)\,dt = Gc(0) + \frac{1}{2CT}\int_0^T dt\int_0^t n_{fork}(t')\,dt' \\ &= Gc(0) + \frac{T}{2C}\langle n_{fork}\rangle - \frac{1}{2CT}\int_0^T n_{fork}(t)t\,dt \\ &= \begin{cases} \left(\frac{C}{2}+D\right)\mu+1, & T \ge C+D, \\ \left(C+2D+\frac{D^2}{2C}\right)\mu+\frac{T}{2C}-\frac{D}{C}, & \frac{C+D}{2} \le T < C+D, \\ \left(2C+4D+\frac{3D^2}{2C}\right)\mu+\frac{9T}{2C}-5\frac{D}{C}-4, & \frac{C+D}{3} \le T < \frac{C+D}{2}, \\ \cdots & \cdots \end{cases} \end{aligned} \tag{A.5}$$

3. The average cell volume is

$$\langle V(t)\rangle = \begin{cases} 3V_0/2 & \text{for linear growth fashion,} \\ V_0/\ln 2 & \text{for exponential growth fashion.} \end{cases} \tag{A.6}$$

4. If the bacteria take linear growth fashion, the initiation volume is

$$V_{ini} = \frac{V(t_{ini})}{n_{ori}(t_{ini})} = \begin{cases} V_0(2-\mu(C+D)), & T \ge C+D, \\ V_0(3-\mu(C+D))/2, & \frac{C+D}{2} \le T < C+D, \\ V_0(4-\mu(C+D))/4, & \frac{C+D}{3} \le T < \frac{C+D}{2}, \\ \cdots & \cdots \end{cases} \tag{A.7}$$

whereas if the bacteria take exponential growth fashion, the initiation volume is

$$V_{ini} = \frac{V(t_{ini})}{n_{ori}(t_{ini})} = 2V_{00}. \tag{A.8}$$

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